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- 1 -

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[Item]	Abstract	1

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[Title of Invention] GLYCOSYLTRANSFERASE, NUCLEIC ACID ENCODING THE GLYCOSYLTRANSFERASE, AND METHOD OF TESTING CANCERATION USING THE NUCLEIC ACID

[Claims]

[Claim1] Anucleicacidhybridizingunderstringentconditions to a nucleotide sequence described in SEQ ID NO: 1 or a

complementary nucleotide sequence thereof.

[Claim 2] The nucleic acid according to Claim 1, wherein the nucleic acid consists of a nucleotide sequence having at least 15 contiguous nucleotides in a nucleotide sequence described in SEQ ID NO: 1 or a complementary nucleotide sequence thereof.

[Claim 3] The nucleic acid according to Claim 2, wherein the nucleic acid consists of a nucleotide sequence described in SEQ ID NO: 1 or a complementary nucleotide sequence thereof.

[Claim 4] The nucleic acid according to any one of Claims 1 to 3, wherein the nucleic acid is a probe or a primer.

[Claim 5] The nucleic acid according to any one of Claims 1 to 4, wherein the nucleic acid is a tumor marker.

[Claim 6] A method of testing canceration of a biological sample, comprising:

- (a) using a nucleic acid according to any one of Claims 1 to 5 to measure the transcription level of the nucleic acid in the biological sample; and

- (b) diagnosing the biological sample as being cancerous when the transcription level of the nucleic acid in the biological sample significantly exceeds that in a normal biological sample as a control.

[Claim 7] The method of testing canceration of a biological sample according to Claim 6, comprising:

- (a) using a nucleic acid according to any one of Claims 1 to 5 as a labeled probe, which is in turn brought into contact with the biological sample under stringent hybridization conditions to measure the transcription level of the nucleic

acid in the biological sample based on a signal from the label of the hybridized nucleic acid; and

(b) diagnosing the biological sample as being cancerous when the transcription level of the nucleic acid in the biological sample significantly exceeds that in a normal biological sample as a control.

[Claim 8] The method of testing the canceration of a biological sample according to Claim 6, comprising:

(a) using a primer according to claim 4 that is labeled to subject a biological sample to nucleic acid amplification and measuring the amount of a resulting nucleic acid amplification product; and

(b) diagnosing the biological sample as being cancerous when the amount of the nucleic acid amplification product significantly exceeds that in a normal biological sample as a control.

[Claim 9] A method of examining the effectiveness of treatment for cancer therapy by use of a nucleic acid according to any one of Claims 1 to 5, comprising:

using a nucleic acid according to any one of Claims 1 to 5 to measure the transcription level of the nucleic acid in a biological sample that has received treatment for cancer therapy and comparing its measurement value with that before the treatment or without the treatment, thereby determining whether the treatment given to the biological sample is effective or not.

[Claim 10] The method according to Claim 9, comprising: using

the biological sample which has already been cancerous and determining that treatment for cancer therapy given to the biological sample is effective when the transcription level of the nucleic acid in the biological sample that has received the treatment is significantly below that before the treatment or without the treatment.

[Claim 11] The method according to Claim 9 or 10, wherein the biological sample is an *in vivo* biological sample from a non-human model animal.

[Claim 12] The method according to any one of Claims 6 to 11, wherein the biological sample is a sample derived from the large intestine or peripheral blood.

[Claim 13] A nucleic acid comprising a nucleotide sequence described in SEQ ID NO: 1, wherein the nucleic acid encodes a glycosyltransferase.

[Claim 14] The nucleic acid according to Claim 13, characterized in that the nucleic acid is DNA.

[Claim 15] A vector comprising a nucleic acid according to Claim 13 or 14.

[Claim 16] A transformant comprising a vector according to Claim 15.

[Claim 17] A polypeptide comprising an amino acid sequence of SEQ ID NO: 2.

[Claim 18] an antibody against a polypeptide comprising an amino acid sequence of SEQ ID NO: 2.

[Detailed Description of the Invention]

[0001]

[Technical Field to which the Invention Belongs]

The present invention relates to a novel nucleic acid, the nucleic acid for testing canceration, and a method of testing the canceration of a biological sample based on a difference in the expression level of the nucleic acid in the biological sample; as well as, to a novel glycosyltransferase and a nucleic acid encoding the glycosyltransferase, and the like.

[0002]

[Background Art]

In recent years, attention has been given to the function of oligosaccharides and glycoconjugates in living bodies. For example, a determinant factor of a blood type is a glycoprotein, and one involved in the function of the nervous system is a glycolipid. Thus, an enzyme having the function of synthesizing an oligosaccharide is a crucially important key for analyzing physiological activities produced by various oligosaccharides.

A N-acetyl-D-glucosamine residue (GlcNAc) and a D-galactose residue (Gal), and the like, in sugar are the components of glycosaminoglycan, while they are sugar residues present in various oligosaccharide structures such as sphingoglycolipids, mucin-type oligosaccharides, and asparagine-linked oligosaccharides (N-linked oligosaccharides). Thus, an enzyme transferring GlcNAc or Gal is a crucially important tool for analyzing the function of oligosaccharides that work in various tissues in living bodies.

[0003]

For example, at least 20 types of N-acetylglucosaminyltransferases having an activity of transferring GlcNAc have been known as shown in Table 1, each of which differs in acceptor substrate specificity (References 1 to 18).

On the other hand, oligosaccharide synthesis is known to be altered with great frequency in canceration and to be correlated with the metastasis and malignancy of cancer (References 30 to 32). Their comprehensive studies actively conducted today, for example, analysis such as expression profiling in a variety of tissues, are also directed to the elucidation of a canceration mechanism, and discussions have often been conducted on the possibility that the canceration mechanism is associated with the expression level of a particular gene. As well known, the test of tumor markers or the like in blood and the identification of the other gene products involved in canceration, and so on, have already been conducted as methods of cancer diagnostic tests. Tumor markers include many antibodies against oligosaccharides. Among others, immunoassay for oncogene products has often been adopted because of its advantage in high sensitivity.

[Table 1]

Table 1 N-acetylglucosaminyltransferases and their substrate specificity

Official name	Abbreviation	Linkage type	Substrate specificity	Reference
N-acetylglucosaminyltransferase-I	GnT-I	β 1-2	Man α 1-3 Man α 1-6 Man α 1-3) Man β 1-4GlcNAc β 1-4G3cNAc β 1-Asn	1
N-acetylglucosaminyltransferase-II	GnT-II	β 1-2	Man α 1-6 (GlcNAc β 1-2Man α 1-3) Man β 1-4GlcNAc β 1-4G3cNAc β 1-Asn	2
N-acetylglucosaminyltransferase-III	GnT-III	β 1-4	GlcNAc β 1-2Man α 1-6 (GlcNAc β 1-2Man α 1-3) Man β 1-4GlcNAc β 1-4G3cNAc β 1-Asn	3
N-acetylglucosaminyltransferase-IV	GnT-IV	β 1-4	GlcNAc β 1-2 (GlcNAc β 1-6) Man α 1-6 (GlcNAc β 1-2Man α 1-3) Man β 1-4GlcNAc β 1-4G3cNAc β 1-Asn	4
N-acetylglucosaminyltransferase-V	GnT-V	β 1-6	GlcNAc β 1-2Man α 1-6 (GlcNAc β 1-2) (GlcNAc β 1-4) Man α 1-3) Man β 1-4GlcNAc β 1-4G3cNAc β 1-Asn	5
N-acetylglucosaminyltransferase-VI	GnT-VI	β 1-4	GlcNAc β 1-2 (GlcNAc β 1-6) Man α 1-6 (GlcNAc β 1-2) (GlcNAc β 1-4) Man α 1-3) Man β 1-4GlcNAc β 1-4G3cNAc β 1-Asn	6
β 1, 3-N-acetylglucosaminyltransferase	iGnT	β 1-3	Gal β 1-4GlcNAc β 1-R	7
β 1, 3-N-acetylglucosaminyltransferase-2	β 3GnT2	β 1-3	Gal β 1-4GlcNAc β 1-R	8
β 1, 3-N-acetylglucosaminyltransferase-3	β 3GnT3	β 1-3	Gal β 1-3GalNAc-0-S/T	8
β 1, 3-N-acetylglucosaminyltransferase-4	β 3GnT4	β 1-3	Gal β 1-4 (GlcNAc β 1-3Gal β 1-4) n-R	8
β 1, 3-N-acetylglucosaminyltransferase-5	β 3GnT5	β 1-3	Gal β 1-4GlcNAc β 1-3Gal β 1-4-Cer	9
β 1, 3-N-acetylglucosaminyltransferase-6	β 3GnT6	β 1-3	GalNAc-0-S/T	10
β 1, 3-N-acetylglucosaminyltransferase-7	β 3GnT7	β 1-3	Gal β 1-4 (GlcNAc β 1-3Gal β 1-4) n-Cer	11
β 1, 3-N-acetylglucosaminyltransferase	Fringe	β 1-3	C2-X-X-G-G- <u>U</u> lc-0) S/T-C3	12
β 1, 6-N-acetylglucosaminyltransferase	iGnT	β 1-6	GlcNAc β 1-3Gal β 1-4GlcNAc β 1-R	13
Core 2 β 1, 6-N-acetylglucosaminyltransferase-I	C2GnT-I	β 1-6	Gal β 1-3GalNAc-0-S/T	14
Core 2 β 1, 6-N-acetylglucosaminyltransferase-II	C2GnT-II	β 1-6	Gal β 1-3GalNAc-0-S/T	15
Core 2 β 1, 6-N-acetylglucosaminyltransferase-III	C2GnT-III	β 1-6	Gal β 1-3GalNAc-0-S/T	16
α 1, 4-N-acetylglucosaminyltransferase	α 4GnT	α 1-4	Gal β 1-3 (Gal β 1-4GlcNAc β 1-6) GalNAc-R	17
peptide β -N-acetylglucosaminyltransferase	OGT	0-	Y-S-D-S-P-S-T-S-T	18

Transferring N-acetylglucosamine to an underlined sugar or amino acid.

[0005]

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[0006]

Table 2

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[0007]

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[0008]

[Problems that the Invention is to Solve]

As described above, the identification of gene products having some involvement in canceration is expected to provide tumor markers useful in cancer diagnosis. If especially a nucleic acid found in a transcript can be used as an indicator for testing canceration, only the identification of a transcript of a particular gene can sufficiently provide an indicator useful in testing canceration, without the need for elucidating the function of its end product, for example, a protein. Especially

the identification of a nucleic acid has advantages which are not found in immunoassay, because it can be performed on a DNA microarray and a nucleic acid even in small amounts can also be quantified after being amplified by PCR.

[0009]

On the other hand, the function of oligosaccharides in living bodies receives attention. However, the analysis of oligosaccharide synthesis in living bodies does not necessarily progress satisfactorily. This is partly because the mechanism of oligosaccharide synthesis and the localization of sugar synthesis in living bodies are not sufficiently elucidated. The analysis of the mechanism of oligosaccharide synthesis requires the analysis of enzymes synthesizing oligosaccharides, especially glycosyltransferases, and analyzing which type of oligosaccharide is generated with the enzyme. Therefore, there also has been a growing demand for finding a novel glycosyltransferase and analyzing its function.

In light of the above-described problems, an object of the present invention is to provide a tumor marker nucleic acid significantly altered in its transcription level following canceration, a nucleic acid for testing canceration that targets the tumor marker nucleic acid, and a method of testing canceration using any of these nucleic acids.

Another object of the present invention is to provide a nucleic acid encoding a novel human glycosyltransferase, and the novel glycosyltransferase in the analysis of a particular gene that receives attention as an indicator of the canceration.

A further alternative object of the present invention is to provide a transformant expressing the nucleic acid in a host cell as well as a method of growing the transformant to isolate the protein.

[0010]

[Means of Solving the Problems]

The present inventors attempted the isolation and purification of a nucleic acid of interest that seems to have high sequence homology based on the nucleotide sequence of a gene of an enzyme similar in action to an enzyme of interest. Specifically, at first, the present inventors conducted a BLAST search using the sequence of a β 1,3-N-acetylglucosaminyltransferase, a glycosyltransferase known in the art, as a query sequence and consequently found a genome sequence (GenBank No. AC011462) as a sequence having homology.

Further, the present inventors successfully cloned a gene encoding the protein by PCR and determined its nucleotide sequence (SEQ ID NO: 1) and predicted amino acid sequence (SEQ ID NO.: 2). A gene having the nucleotide sequence of SEQ ID NO: 1 was designated as a G9 gene, and a protein having the amino acid sequence of SEQ ID NO: 2 was designated as a G9 protein. The present inventors have thereby completed the present invention by finding out that a protein encoded by the nucleic acid is a novel glycosyltransferase and that the presence or absence of the expression of the nucleic acid or its expression level in cancerous tissues differs from that in normal tissues.

[0011]

The present invention relates to a nucleic acid hybridizing under stringent conditions to a nucleotide sequence described in SEQ ID NO: 1 or a complementary nucleotide sequence thereof.

Preferably, the nucleic acid of the present invention consists of a nucleotide sequence having at least 15 contiguous nucleotides in a nucleotide sequence described in SEQ ID NO: 1 or a complementary nucleotide sequence thereof.

The nucleic acid of the present invention is typically a probe or a primer. The nucleic acid of the present invention can also be a tumor marker..

[0012]

The present invention also relates to a method of testing the canceration of a biological sample, comprising:

(a) using any of the above-described nucleic acids to measure the transcription level of the nucleic acid in a biological sample; and

(b) diagnosing the biological sample as being cancerous when the transcription level of the nucleic acid in the biological sample significantly exceeds that in a normal biological sample as a control.

According to a preferred aspect of the testing method by the present invention, the method of testing the canceration of a biological sample comprises:

(a) using any of the above-described nucleic acids as a labeled probe, which is in turn brought into contact with a biological sample under stringent hybridization conditions to

measure the transcription level of the nucleic acid in the biological sample based on a signal from the label of the hybridized nucleic acid; and

(b) diagnosing the biological sample as being cancerous when the transcription level of the nucleic acid in the biological sample significantly exceeds that in a normal biological sample as a control.

[0013]

According to another preferred aspect of the testing method by the present invention, the method of testing the canceration of a biological sample comprises:

(a) using the above-described primer that is labeled to subject a biological sample to nucleic acid amplification and measuring the amount of a resulting nucleic acid amplification product; and

(b) diagnosing the biological sample as being cancerous when the amount of the nucleic acid amplification product significantly exceeds that in a normal biological sample as a control.

[0014]

According to a further aspect of the testing method by the present invention, the effectiveness of treatment for cancer therapy can be examined by use of the nucleic acid of the present invention.

The method of examining the effectiveness of treatment for cancer therapy by the present invention is a method comprising:

using any of the nucleic acids according to the present

invention to measure the transcription level of the nucleic acid in a biological sample that has received treatment for cancer therapy and comparing its measurement value with that before the treatment or without the treatment, thereby determining whether the treatment given to the biological sample is effective or not.

[0015]

A preferred aspect of the method of examining the effectiveness of treatment by the present invention encompasses a method comprising: using the biological sample which has already been cancerous, and determining that treatment for cancer therapy given to the biological sample is effective if the transcription level of the nucleic acid in the biological sample that has received the treatment is significantly below that before the treatment or without the treatment.

The biological sample to which the method of examining the effectiveness of treatment can be applied includes an *in vivo* biological sample from a non-human model animal as well as an *in vitro* biological sample derived from a tissue, a cell, or the like (including a human tissue or cell, or the like). Alternatively the biological sample to which each of the above-described methods according to the present invention can be applied is typically a sample derived from the large intestine or peripheral blood.

[0016]

In other aspects of the present invention, the nucleotide sequence of SEQ ID NO: 1 has 31% homology to those of known genes

a human $\beta 1,3$ GlcNAc transferase 2 and a $\beta 1,3$ Gal transferase 6, and a conserved motif therein is close to that in a $\beta 1,3$ Gal transferase. The nucleotide sequence has 60% homology to that of a murine $\beta 1,3$ GlcNAc transferase 1. The predicted amino acid sequence of SEQ ID NO: 2 has a hydrophobic transmembrane region characteristic of a glycosyltransferase at its N terminus.

[0017]

From these points of view, the nucleic acid sequence of SEQ ID NO: 1 presumably encodes a novel human glycosyltransferase that transfers an N-acetyl-D-glucosamine residue to synthesize an oligosaccharide through $\beta 1,3$ -linkage. On the other hand, from the experimental findings, the nucleic acid has an possibility to encode a novel human glycosyltransferase that transfers an N-acetyl-D-galactosamine residue and synthesizes sugar chain through $\beta 1,3$ -linkage.

[0018]

A protein consisting of the amino acid sequence of SEQ ID NO: 2 has the activity of the above novel glycosyltransferase. Providing an amino acid sequence of this novel protein and a nucleic acid encoding would make a contribution towards satisfying diverse needs for them in the art.

[0019]

That is, the present invention also relates to a glycosyltransferase protein from a sugar donor substrate to a sugar acceptor substrate and relates to a nucleic acid encoding the protein. The typical sugar donor substrate is UDP-GlcNAc, and at least a Gal $\beta 1,4$ GlcNAc carbohydrate residue is the acceptor

substrate.

[0020]

In an aspect of the nucleic acid encoding a glycosyltransferase protein of the present invention, the nucleic acid is DNA. In a further alternative aspect, the present invention also relates to a vector comprising the nucleic acid as described above as well as a transformant comprising the vector. The present invention further relates to a method of producing the glycosyltransferase, comprising: growing the above-described transformant comprising the above described vector and expressing the protein to collect the protein from the transformant.

In a still alternative aspect, the present invention can provide a glycosyltransferase protein comprising an amino acid sequence of SEQ ID NO: 2 and an antibody recognizing the glycosyltransferase protein.

The finding that the nucleic acid of the present invention encodes a novel glycosyltransferase protein suggests that the expression level of the glycosyltransferase protein in a cancer tissue exceeds that in a normal tissue. Accordingly, it may be possible to test the canceration of a biological sample by detecting or quantifying the protein of the present invention that is expressed in the biological sample to compare its result with that of a normal biological sample as a control.

[0021]

Thus, the present invention also relates to a method of testing the canceration of a biological sample, comprising the

steps of:

(a) detecting or quantifying the novel glycosyltransferase protein of the present invention in a biological sample; and

(b) diagnosing the biological sample as being cancerous when the quantified value of the glycosyltransferase protein in the biological sample significantly exceeds that of the glycosyltransferase protein in a normal biological sample as a control.

Here, the use of an antibody specifically recognizing a glycosyltransferase protein is exemplified for detecting the novel glycosyltransferase protein.

[0022]

[Preferred Mode of Carrying Out the Invention]

Hereinafter, the present invention will be described in detail in accordance with embodiments of the present invention.

(1) Nucleic acid of the present invention involved in canceration

The present inventors have found that the canceration of a normal tissue, for example, a human large intestine tissue, without the expression of a nucleic acid having a nucleotide sequence described in SEQ ID NO: 1 is confirmed to cause the nucleic acid to be expressed therein, and that a normal tissue, for example, peripheral blood from a patient with colorectal cancer, in which a nucleic acid having a nucleotide sequence described in SEQ ID NO: 1 is generally expressed has a significant increase in the expression level of the nucleic acid as compared to that in a normal individual.

Thus, a nucleic acid consisting of the nucleotide sequence

of SEQ ID NO: 1 or a complementary sequence thereof is worthy of note as a tumor marker useful in examination for a transcript in a biological sample. According to the present invention, a nucleic acid capable of specifically hybridizing under stringent conditions to this tumor marker nucleic acid is provided.

[0023]

A primer or probe according to the present invention is typically a natural DNA fragment derived from a nucleic acid having a nucleotide sequence of SEQ ID NO: 1, a synthetic DNA fragment designed to have a nucleotide sequence of SEQ ID NO: 1, or any of complementary strands thereof.

Especially the tumor marker nucleic acid was detected via a BLAST search and is transcribed as mRNA encoding a structural gene. In general, its full-length ORF or a portion thereof can be present in a sample. From this point of view, using the nucleic acid as a targeting primer or probe, a desired target sequence can be selected from across the ORF of the nucleotide sequence of SEQ ID NO: 1. The primer or probe of the present invention can be a partial sequence in the nucleotide sequence of SEQ ID NO: 1.

Using the primer or probe as described above, the target nucleic acid in a biological sample can be detected and/or quantified as described below. Because a genomic sequence or the like can be targeted, the nucleic acid of the present invention can also be provided as an antisense primer for medical research or gene therapy.

[0024]

Probe of the present invention

When the nucleic acid of the present invention is used as a probe, the nucleic acid is an oligonucleotide with 15 bases or more, preferably 20 bases or more, selected from the nucleotide sequence of SEQ ID NO: 1 or a complementary strand thereof, or alternatively cDNA with a maximum length of a full-length ORF region (i.e., 1191 bases: nucleotide Nos. 1 to 1191) in the nucleotide sequence of SEQ ID NO: 1 or a complementary strand thereof.

In particular, the probe of the present invention is widely useful as a reagent or a diagnostic agent for medical research. Considering that a nucleic acid having an exceedingly large molecular weight is generally difficult to handle, a preferred base length of the probe is exemplified by 50 to 500 bases, more preferably 60 to 300 bases.

Depending on, for example, the base length or hybridization conditions adopted, an oligonucleotide probe having a relatively short strand can function as a probe even if there is a mismatch on the order of one or several bases, especially one or two bases, between the oligonucleotide probe and the nucleotide sequence of SEQ ID NO: 1 or the complementary nucleotide sequence thereof. A cDNA probe having a relatively long strand can function as a probe even if there is a mismatch of 50% or less, preferably 20% or less, between the cDNA probe and the nucleotide sequence of SEQ ID NO: 1 or the complementary nucleotide sequence thereof.

[0025]

Alternatively, when the nucleic acid of the present invention is a synthetic oligonucleotide, the number of bases therein is 15 bases or more, preferably 20 bases or more. Depending on the base length or hybridization conditions adopted, the synthetic oligonucleotide can function as a probe even if there is a mismatch on the order of one or several bases, especially one or two bases, between the synthetic oligonucleotide and the nucleotide sequence described in SEQ ID NO: 1 or the complementary nucleotide sequence thereof.

It should be understood that the oligonucleotide probe according to the present invention having 15 bases in length could specifically hybridize under stringent conditions to the target nucleic acid. Those skilled in the art can select a suitable partial sequence with at least 15 bases from the nucleotide sequence of SEQ ID NO: 1 according to various strategies concerning oligonucleotide probe design known in the art. Moreover, information from an amino acid sequence of SEQ ID NO: 2 would be helpful in selecting a unique sequence likely to be suitable as a probe.

[0026]

"Under stringent conditions" used herein means hybridization under moderately or highly stringent conditions. Specifically, the moderately stringent conditions are based on, for example, the length of DNA and can readily be determined by those having ordinary skill in the art. Basic conditions are shown in Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd ed., Vol. 1, 7.42-7.45 Cold Spring Harbor Laboratory

Press, 2001 and include, for a nitrocellulose filter, the use of hybridization conditions comprising a prewashing solution containing 5×SSC, 0.5% SDS, 1.0 mM EDTA (pH: 8.0) and a hybridization solution containing approximately 50% formamide, 2×SSC to 6×SSC at approximately 40 to 50°C (or other similar hybridization solutions such as a Stark's solution in approximately 50% formamide at approximately 42°C), and washing conditions comprising 0.5×SSC, 0.1% SDS at approximately 60°C. The highly stringent conditions are also based on, for example, the length of DNA and can readily be determined by those skilled in the art. In general, such conditions include hybridization and/or washing at a temperature higher than that of the moderately stringent conditions and/or a salt concentration lower than that of the moderately stringent conditions and are defined by involving, for example, the hybridization conditions as described above and washing in 0.2×SSC, 0.1% SDS at approximately 68°C. Those skilled in the art would appreciate that a temperature and a salt concentration of a washing solution is optionally adjustable according to factors such as the length of a probe.

[0027]

As described above, those skilled in the art can readily find and practice moderately or highly stringent conditions suitable for a selected probe, based on common general technical knowledge in various probe design methods and hybridization conditions known in the art as well as empirical rules that would be obtained through experimental means usually used.

The probe of the present invention includes a labeled probe attached with a label such as fluorescent, radioactive, and biotin labels, in order to detect or confirm the probe hybridized with the target sequence. One example of the labeled probe according to the present invention is an oligonucleotide consisting of a nucleotide sequence of SEQ ID NO: 6 (which corresponds to a complementary strand of a strand from nucleotide Nos. 485 to 502 in SEQ ID NO: 1). This labeled probe can be used for confirming or quantifying a PCR product of the target nucleic acid. The labeled probe of the present invention may also be integrated in a diagnostic DNA probe kit or the like or may be immobilized on a chip such as a DNA microarray.

[0028]

Primer of the present invention

When the nucleic acid of the present invention is used as a primer, the nucleic acid is an oligonucleotide. Specifically, two regions are selected from the ORF region in the nucleotide sequence of SEQ ID NO: 1 to satisfy the following conditions:

1) each of the regions has a length of 15 bases or more, preferably 18 bases or more, more preferably 21 bases or more and no longer than 50 bases; and

2) each of the regions has a G+C content of 40 to 70%. Single-stranded DNAs having the same nucleotide sequences as those of the two selected regions or nucleotide sequences complementary to those of the regions may be produced, or otherwise the single-stranded DNAs modified to maintain the binding specificity for the nucleotide sequences may be produced.

Preferably, the primer of the present invention has a sequence completely complementary to a partial sequence in the ORF region of SEQ ID NO: 1 and however, may have a one- or two-base mismatch.

One example of a pair of primers according to the present invention is a pair of an oligonucleotide consisting of a nucleotide sequence described in SEQ ID NO: 4 (which corresponds to a complementary strand of a strand from nucleotide Nos. 450 to 469 in SEQ ID NO: 1) and an oligonucleotide consisting of a nucleotide sequence described in SEQ ID NO: 5 (which corresponds to a complementary strand of a strand from nucleotide Nos. 531 to 549 in SEQ ID NO: 1).

A probe selected from the nucleotide sequence positioned between a pair of primers used can be employed for quantifying the target nucleic acid amplified by PCR. One example of a labeled probe for detecting a PCR product is an oligonucleotide consisting of a nucleotide sequence described in SEQ ID NO: 6 (which corresponds to a complementary strand of a strand from nucleotide Nos. 485 to 502 in SEQ ID NO: 1).

[0029]

(2) Method of testing canceration according to the present invention

According to a method of testing canceration of the present invention, the transcription level of the target nucleic acid in a transcript of a biological sample can be measured. Its measurement result is compared with a result from a normal biological sample as a control. If a significant difference lies between those results, the biological sample can be

diagnosed as being a cancerous tissue.

In this testing method, a threshold normalized in advance on the basis of known data concerning a normal biological sample may be used as a detection result for a normal tissue used as a control. For example, when a normal tissue is not obtained from an identical patient as in the test of peripheral blood, comparison with the average of values measured in normal individuals is performed.

As used herein, the significant difference to be diagnosed as being cancerous means that the substantial presence (i.e., significant concentration) of the target nucleic acid in a subject tissue is confirmed if the target nucleic acid is expressed in a normal tissue as in, for example, peripheral blood from a patient with colorectal cancer, or that the concentration of the nucleic acid in a subject tissue significantly exceeds that in a normal tissue and preferably the nucleic acid in the subject tissue is not less than 1.5 times, preferably 2 times, by concentration greater than that in the normal tissue when the target nucleic acid is generally expressed in a normal tissue as in, for example, a colorectal cancer tissue.

The method of testing canceration according to the present invention typically involves a hybridization assay and a PCR method.

[0030]

Hybridization assay

Examples of a hybridization assay that can be used in the present invention include various hybridization assays well

known to those skilled in the art such as a southern blot, northern blot, dot blot, or a colony hybridization technique for a transcript extracted from a biological sample.

Depending on the transcription amount of the target nucleic acid or a difference from a normal tissue, a testing method known in the art including a quantitative hybridization assay such as dot blot or colony hybridization used alone or in combination with immunoassay may be employed when the quantification of the target nucleic acid or an increase in detection level is required.

According to the typical hybridization assay, a subject nucleic acid extracted from a biological sample or an amplification product thereof is immobilized on a solid phase and hybridized under stringent conditions to a labeled probe to measure the label bound to the solid phase after washing.

Every method known to those skilled in the art can be applied to the extraction and purification of a transcript from a biological sample. That purified from a biological sample and subjected to the hybridization assay is typically cDNA from the whole transcript of the biological sample. However, when canceration is determined only by the substantial detection of the target nucleic acid (i.e., when no target nucleic acid appears to be expressed in a normal tissue), the use of a testing method such as in situ hybridization in no need of the purification or the like of a transcript would be practical for a subject tissue in clinical tests.

[0031]

Testing method by nucleic acid amplification

On the basis of "the nucleotide sequence of the nucleic acid of the present invention", those skilled in the art can appropriately create primers based on nucleotide sequences positioned at both ends of the nucleic acid of the present invention or a partial region of interest thereof to be prepared, and readily amplify and prepare the region of interest by nucleic acid amplification reaction (e.g., PCR) using the primers.

As used herein, examples of the nucleic acid amplification reaction include reaction requiring thermal cycles such as polymerase chain reaction (PCR) [Saiki R.K., et al., Science, 230, 1350-1354 (1985)], ligase chain reaction (LCR) [Wu D.Y., et al., Genomics, 4, 560-569 (1989); Barringer K.J., et al., Gene, 89, 117-122 (1990); Barany F., Proc. Natl. Acad. Sci. USA, 88, 189-193 (1991)], and transcription-based amplification [Kwoh D.Y., et al., Proc. Natl. Acad. Sci. USA, 86, 1173-1177 (1989)], and isothermal reaction such as strand displacement amplification (SDA) [Walker G.T., et al., Proc. Natl. Acad. Sci. USA, 89, 392-396 (1992); Walker G.T., et al., Nuc. Acids Res., 20, 1691-1696 (1992)], self-sustained sequence replication (3SR) [Guatelli J.C., Proc. Natl. Acad. Sci. USA, 87, 1874-1878 (1990)], and Q β replicase system [Lizardi et al., BioTechnology 6, p. 1197-1202 (1988)]. For example, Nucleic Acid Sequence-Based Amplification (NASBA) reaction described in European Patent No. 0525882, which employs the competitive amplification of a target nucleic acid with a mutant sequence is also available. Preferably, the nucleic acid amplification

reaction is the PCR method.

[0032]

The target nucleic acid in a transcript can be detected using PCR method with, for example, a pair of primers of the present invention selected from the target nucleic acid. In general, the nucleic acid amplification method in itself, such as PCR, is well known in the art and is readily carried out because a reagent kit and an apparatus for the nucleic acid amplification method are commercially available.

When the primer pair of the present invention is used to carry out a nucleic acid amplification method by PCR with the subject nucleic acid as a template, the subject nucleic acid present in a sample is amplified while no amplification takes place in a sample without the subject nucleic acid. Therefore, whether or not the subject nucleic acid is present in the sample can be determined by confirming the presence of an amplification product, and the transcription level of the subject nucleic acid, that is, the concentration thereof can also be determined by quantifying an amplification product. PCR cycles when repeated a predetermined number of times would amplify the subject nucleic acid to a desired concentration. The nucleic acid in a normal tissue can also be measured in a similar way. A nucleic acid of a gene extensively and generally present in an identical tissue or the like, for example, a nucleic acid encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or β -actin may be used as a control.

[0033]

The subject nucleic acid may be the whole mRNA as a transcript extracted from a biological sample such as a subject tissue or cell or may be the whole cDNA reverse-transcribed from the mRNA. When mRNA as the subject nucleic acid is amplified, a NASBA method (3SR method, TMA method) using the above-described pair of primers may be adopted. The NASBA method in itself is well known and can readily be practiced using the pair of primers because a kit for the NASBA method is commercially available.

An amplification product obtained by PCR method can be detected or quantified by procedures where the reaction solution after amplification is subjected to electrophoresis and the resulting bands are stained with ethidium bromide or the like or procedures where the amplification product after electrophoresis is immobilized on a solid phase such as a nylon membrane and hybridized to a labeled probe specifically hybridizing to the subject nucleic acid, for example, a labeled probe described in SEQ ID NO: 6, to detect the label after washing.

When the "concentrations" of the target nucleic acids are compared between a subject tissue and a normal tissue, it is preferable to use a quantitative PCR method including a RT-PCR or quantitative real-time PCR method for kinetic analysis. Because the target nucleic acid previously purified is mRNA, a quantitative real-time RT-PCR method is particularly preferred. However, the quantification of the nucleic acid in the present testing method is not limited to the quantitative PCR method, and other DNA quantification methods known in the art such as northern blot, dot blot, and a DNA microarray using the

above-described probe for a PCR product are applicable.

It is also possible to quantify the amount of the target nucleic acid in a sample by carrying out quantitative RT-PCR using quencher and reporter fluorescent dyes. Because especially a kit for the quantitative RT-PCR is commercially available, the quantitative RT-PCR can readily be carried out. In addition, it is also possible to semi-quantify the target nucleic acid based on the strength of electrophoretic bands.
[0034]

Testing method for effectiveness of cancer therapy

The above-described method of testing canceration according to the present invention can also be employed as a method of examining the effectiveness of cancer therapy. Subjects to be examined are the effectiveness of treatment for which the effectiveness of cancer cure should be examined as well as the effectiveness of treatment given to a cancerous cell or tissue or a tumor tissue or the like obtained from a model animal for experimental carcinogenesis. Such treatment includes every prescription such as radiotherapy in addition to the administration of an anticancer agent. The treatment is given to a cancerous biological sample or to the focus of an experimental model animal.

According to a method of examining the effectiveness of cancer therapy according to the present invention, the transcription level of the target nucleic acid in a biological sample that has received treatment of interest is compared with that before the treatment or without the treatment.

Alternatively, the transcription level may also be followed up after the treatment. The treatment can be assessed as being effective for cancer therapy if by using it the treatment causes the transcription level to be significantly reduced or causes an intentional rise in the transcription level to be significantly suppressed.

Such examination includes determination about whether a candidate substance of an anticancer agent given to a cancerous tissue is effective or not, especially whether the candidate substance is effective or not for a lesion tissue or the like in an experimental model animal, and determination about whether a novel candidate anticancer agent is effective or not for a patient with cancer. To the contrary, assessment of whether carcinogenicity is suppressed or not in an experimental model animal designed to develop cancer, that is, whether an expected rise in the transcription level is significantly suppressed or not is also targeted.

[0035]

In the present specification, the "transcription level" or "transcription amount" of a nucleic acid refers to the abundance of the nucleic acid derived from a transcript in a fixed amount of a biological sample. Because a nucleic acid can be amplified for quantification or the signal level of its label can be amplified, the amount of the nucleic acid measured can also be expressed as an amplified amount or an amplified signal level.

[0036]

In the present specification, a "subject nucleic acid" or "target nucleic acid" includes not only mRNA and siRNA but also every type of nucleic acid obtained with mRNA as a template, regardless of *in vivo* or *in vitro* origin.

In the present specification, a "biological sample" refers to an organ, a tissue, and a cell as well as an experimental animal-derived organ, tissue, and cell, etc., and is preferably a tissue. The esophagus, the stomach, the pancreas, the liver, the kidney, the duodenum, the small intestine, the large intestine, the rectum, the colon, and peripheral blood are concretely exemplified. Preferred are the large intestine, the rectum, the colon, and peripheral blood, and more preferred are the large intestine and peripheral blood. The term "measurement" used herein encompasses any of detection, amplification, quantification, and semi-quantification. The application of the nucleic acid of the present invention also includes gene therapy.

[0037]

The testing method of the present invention is a method of testing the canceration of a biological sample as described above. The phrase "testing canceration" used herein includes testing to determine whether the biological sample develops cancer or not as well as testing to determine whether malignancy is high or not, and can be applied to diagnosis, therapy, and so on, for cancer in medical care. The term "cancer" used herein typically refers to the entire spectrum of malignant tumors and includes disease conditions caused by a malignant tumor. The

testing method of the present invention is suitable for testing, but not limited to, cancer of the esophagus, gastric cancer, cancer of the pancreas, cancer of the liver, renal cancer, duodenal cancer, cancer of the small intestine, colorectal cancer, cancer of the rectum, cancer of the colon, and peripheral blood. Preferred are colorectal cancer, cancer of the rectum, and cancer of the colon, and more preferred is colorectal cancer.

[0038]

(2) Nucleic acid of the present invention encoding novel glycosyltransferase

Based on the finding of the nucleic acid described above, the present invention also provides a nucleic acid encoding a full-length novel glycosyltransferase protein or a fragment thereof.

The nucleic acid of the present invention encoding a novel glycosyltransferase is a nucleic acid consisting of a nucleotide sequence described in SEQ ID NO: 1 or a complementary nucleotide sequence thereof. Further, a nucleic acid encoding an amino acid sequence identical to any amino acid sequence encoded by those nucleic acids because of codon degeneracy is also encompassed by the present invention. As previously described, these nucleic acids are nucleic acids suitable for use in, for example, the method of testing canceration.

[0039]

The nucleic acid of the present invention encoding the novel glycosyltransferase includes both single-stranded and double-stranded DNAs and also includes RNA complements thereof.

Examples of the DNA include naturally-derived DNA, recombinant DNA, DNAs chemically bonded together, DNA amplified by PCR, and combinations thereof. However, DNA is preferred in light of its stability at the time of preparing a vector and a transformant.

The nucleic acid of the present invention may be prepared by, for example, procedures below.

At first, BLAST search is carried out using a sequence of a β 1,3-N-acetylglucosaminyltransferase, a glycosyltransferase known in the art, as a query. As a result, genome sequence (GenBank No. AC011462) can be found as a sequence having a homology. The complementary sequence of the nucleic acid or a portion thereof is utilized to carry out nucleic acid amplification reaction from a cDNA library or the like according to a standard method using a basic genetic engineering approach such as hybridization and nucleic acid amplification reaction, thereby allowing the preparation of the nucleic acid of the present invention. Because, for example, an approximately 1.1-kbp DNA fragment is obtained as a PCR product, this fragment can be separated by a method such as agarose gel electrophoresis, which screens DNA fragments according to their molecular weights, and then isolated according to a standard method such as a method for cutting out a certain band.

[0040]

Because its amino acid sequence of the nucleic acid is expected, from a predicted amino acid sequence (SEQ ID NO: 2), to have a transmembrane region at the N-terminus, the nucleic acid of the present invention that encodes a solubilized form

of a polypeptide can also be obtained by preparing a region of a nucleotide sequence encoding a polypeptide without the transmembrane region.

[0041]

A homologous nucleic acid cloned using the hybridization and nucleic acid amplification reaction described above has at least 50% identity, preferably at least 60% identity, more preferably at least 70% or more identity, even more preferably at least 80% identity, still more preferably at least 90 % or more identity, most preferably at least 95% identity, to the nucleotide sequence described in SEQ ID NO: 1.

[0042]

It is possible to determine the percentage of identity by visual inspection and mathematical calculation. Or otherwise, the percentage of identity of two nucleic acid sequences can be determined by using the GAP computer program, version 6.0 described in Devereux et al., Nucl. Acids Res. 12: 387, 1984 and available from the University of Wisconsin Genetics Computer Group (UWGCG) to compare sequence information. Preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identity and 0 for non-identity) for nucleotide and the weighted comparison matrix of Gribskov and Burgess, Nucl. Acids Res. 14: 6745, 1986 as described in Schwartz and Dayhoff, eds., Atlas of Protein Sequence and Structure, pp. 353-358, National Biomedical Research Foundation, 1979; (2) a penalty of 3.0 for each gap and an additional penalty of 0.10 for each symbol in each gap;

and (3) no penalty for end gaps. Other programs of sequence comparison used by those skilled in the art are also available.

[0043]

(3) Vectors and transformants of the present invention

According to the present invention, a recombinant vector containing the above-described nucleic acid that has been isolated is provided. An example of a method for incorporating a DNA fragment of the nucleic acid into a vector such as a plasmid includes a method described in Sambrook, J. et al., Molecular Cloning, A Laboratory Manual (3rd edition), Cold Spring Harbor Laboratory, 1.1 (2001). Conveniently, a commercially-available ligation kit (e.g., from TAKARA SHUZO) can also be used. The recombinant vector (e.g., the recombinant plasmid) thus obtained is introduced into a host cell (e.g., E. coli DH5 α , TB1, LE392, or XL-LE392 or XL-1Blue).

[0044]

A method for introducing a plasmid into a host cell includes a calcium chloride method or calcium chloride/rubidium chloride method, an electroporation method, an electroinjection method, a method by chemical treatment such as PEG, and a method using a gene gun or the like, described in Sambrook, J. et al., Molecular Cloning, A Laboratory Manual (3rd edition), Cold Spring Harbor Laboratory, 16.1 (2001).

The vector can be prepared simply by ligating a desired gene with a vector for recombination (e.g., plasmid DNA) available in the art according to a standard method. Concrete examples of the vector used include, but are not limited to,

pDONR201, pBluescript, pUC18, pUC19, and pBR322 as a plasmid derived from E. coli.

Those skilled in the art can appropriately select restriction ends to be compatible with an expression vector. Those skilled in the art can appropriately select an expression vector suitable for a host cell desired to express the enzyme of the present invention. Thus, it is preferred that the expression vector according to the present invention should be constructed so that regions involved in gene expression (such as promoter, enhancer, and operator regions) are properly arranged to allow the expression of the nucleic acid in a host cell of interest and the nucleic acid is properly expressed. The construction of the expression vector can also employ a Gateway system (Invitrogen) which does not require restriction treatment and ligation procedures. The Gateway system is a system utilizing site-specific recombination, which allows the cloning of a PCR product while maintaining its orientation and also allows the subcloning of a DNA fragment into an expression vector properly modified. Specifically, an entry clone is created from a PCR product and a donor vector with a BP clonase, a site-specific recombinase, and the PCR product is then transferred to a destination vector that is capable of undergoing recombination between the destination vector and the entry clone through another recombinase LR clonase, thereby preparing an expression clone compatible with an expression system. One of the features of the Gateway system is that, once an entry clone is initially created, no laborious subcloning step having

procedures with restriction enzymes and ligases is required.
[0045]

The type of an expression vector is not particularly limited as long as the expression vector has the function of expressing a desired gene in a variety of prokaryotic and/or eukaryotic host cells to produce a desired protein. Examples of a preferred expression vector include: pQE-30, pQE-60, pMAL-C2, pMAL-p2, and pSE420 for *E. coli*; pYES2 (*Saccharomyces*), and pPIC3.5K, pPIC9K, and pA0815 (*Pichia*) for yeast; and pFastBac, pBacPAK8/9, pBK283, pVL1392, and pBlueBac4.5 for insects.

The incorporation of the expression vector of the present invention into a host cell can give a transformant. The host cell may be a eukaryotic cell (such as a mammalian cell, yeast, and an insect cell) or may be a prokaryotic cell (such as *E. coli* and *B. subtilis*). A host cell for obtaining the transformant of the present invention is not particularly limited and may also be a cultured cell derived from humans (e.g., HeLa, 293T, and SH-SY5Y), mice (e.g., Neuro2a, NIH3T3), and so on. Any of these are known in the art and are commercially available (e.g., from DAINIPPON PHARMACEUTICAL) or available from public research institutes (e.g., RIKEN Cell Bank). Alternatively, an embryo, an organ, a tissue, or a non-human individual may also be used.
[0046]

Incidentally, the nucleic acid of the present invention is a nucleic acid found in a human genome library. Therefore, in the present invention, by using a eukaryotic cell as a host cell for the transformant of the present invention, the "nucleic

acid of the present invention" having a property close to that of a natural one (e.g., morphology having the addition of an oligosaccharide) will be obtained. Thus, it is preferable to select a eukaryotic cell, especially a mammalian cell, as the "host cell". The mammalian cell is concretely exemplified by a mouse-derived cell, and an animal cell is exemplified by a mouse-derived, *Xenopus laevis*-derived, rat-derived, hamster-derived, monkey-derived, or human-derived cell or cultured cell lines established from those cells. *E. coli*, yeast, or an insect cell used as a host cell is concretely exemplified by DH5 α , M15, JM109, and BL21 (*E. coli*); INVSc1 (*Saccharomyces*), and GS115 and KM71 (*Pichia*) (yeast); and Sf21, BmN4, and silkworm larva (insect cell).

When a bacterium, especially *E. coli*, is used as the host cell, an expression vector is generally composed of at least a promoter/operator region, an initiation codon, a gene encoding a desired protein, a termination codon, a terminator, and a replicable unit.

[0047]

When yeast, a plant cell, an animal cell, or an insect cell is used as a host cell, it is preferred that an expression vector should generally contain at least a promoter, an initiation codon, a gene encoding a desired protein, a termination codon, and a terminator. For example, DNA encoding a signal peptide, an enhancer sequence, 5'- and 3'-untranslated regions of a desired gene, a selective marker region, or a replicable unit may optionally be contained therein.

In the vector of the present invention, the preferred initiation codon is exemplified by a methionine codon (ATG). Moreover, the termination codon is exemplified by a termination codon regularly used (e.g., TAG, TGA, and TAA).

The replicable unit means DNA having the ability to replicate its total DNA sequence in a host cell and includes a natural plasmid, an artificially-modified plasmid (plasmid prepared from a natural plasmid), and a synthetic plasmid. The preferred plasmid includes: a plasmid pQE30, pET, or pCAL, or artificially-modified products thereof (DNA fragments obtained by treating pQE30, pET, or pCAL with an appropriate restriction enzyme) for *E. coli*; a plasmid pYES2 or pPIC9K for yeast; and a plasmid pBacPAK8/9 for an insect cell.

[0048]

Any of those usually used by those skilled in the art such as enhancer and terminator sequences each derived from SV40 can be used as enhancer and terminator sequences.

Any of those usually used can be used as a selective marker according to a standard method. An example thereof, includes a gene resistant to an antibiotic such as tetracycline, ampicillin, or kanamycin or neomycin, hygromycin, or spectinomycin.

[0049]

The expression vector can be prepared by consecutively and circularly ligating at least the above-described promoter, initiation codon, gene encoding a desired protein, termination codon, and terminator region with an appropriate replicable unit.

On this occasion, an appropriate DNA fragment (e.g., a linker, other restriction sites) can be used, if desired, according to a standard method such as digestion with a restriction enzyme and ligation using a T4 DNA ligase.

[0050]

The introduction [transformation (transfection)] of the expression vector of the present invention into a host cell can be carried out using a method conventionally known in the art.

The expression vector can be transformed into, for example, a bacterium (such as *E. coli* and *Bacillus subtilis*) by, for example, a method of Cohen et al. [Proc. Natl. Acad. Sci. USA, 69, 2110 (1972)], a protoplast method [Mol. Gen. Genet., 168, 111 (1979)], and a competent method [J. Mol. Biol., 56, 209 (1971)], into *Saccharomyces cerevisiae* by, for example, a method of Hinnen et al. [Proc. Natl. Acad. Sci. USA, 75, 1927 (1978)] and a lithium method [J. B. Bacteriol., 153, 163 (1983)], into a plant cell by, for example, a leaf disk method [Science, 227, 129 (1985)] and an electroporation method [Nature, 319, 791 (1986)], into an animal cell by, for example, a method of Graham [Virology, 52, 456 (1973)], and into an insect cell by, for example, a method of Summer et al. [Mol. Cell Biol., 3, 2156-2165 (1983)], respectively.

[0051]

(4) Isolation and purification of enzyme protein according to the present invention

In recent years, an approach in which a transformant is cultured and grown and a substance of interest is isolated and

purified from the cultured and grown products has been established as a genetic engineering approach.

The enzyme protein according to the present invention can be expressed (produced), for example, by culturing a transformant containing the expression vector prepared as described above in a nutrient medium. Preferably, the nutrient medium contains a carbon source, an inorganic nitrogen source, or an organic nitrogen source necessary for the growth of a host cell (transformant). The carbon source is exemplified by glucose, dextran, soluble starch, sucrose, and methanol. The inorganic nitrogen source or organic nitrogen source is exemplified by ammonium salts, nitrates, an amino acid, corn steep liquor, peptone, casein, a meat extract, soy bean cake, and a potato extract. The nutrient medium may also contain other nutrients (e.g., an inorganic salt (e.g., NaCl, calcium chloride, sodium dihydrogenphosphate, and magnesium chloride), vitamins, an antibiotic (e.g., tetracycline, neomycin, ampicillin, and kanamycin)), if desired. Culture is carried out by a method known in the art. Such culture conditions as temperature, pH of a medium, and culture time are appropriately selected to produce the protein according to the present invention in large amounts.

[0052]

The protein according to the present invention can be acquired from a cultured product obtained by the culture described above, as follows: when the protein according to the present invention is accumulated within a host cell, the host

cell is collected by a procedure such as centrifugation and filtration and then suspended in an appropriated buffer (e.g., a buffer such as Tris, phosphate, HEPES, and MES buffers having a concentration of approximately 10 to 100 mM; pH is preferably in the range of 5.0 to 9.0, which differs depending on a buffer used), followed by the disruption of the cell by a method suitable for the host cell used to obtain contents of the host cell by centrifugation; whereas, when the protein according to the present invention is secreted to the outside of a host cell, the host cell is separated from a medium by a procedure such as centrifugation and filtration to obtain a culture filtrate. A host cell disruption solution or culture filtrate can be subjected to the isolation and purification of the protein either directly or after subjecting to ammonium sulfate precipitation and dialysis. A method of isolating and purifying the protein can include the following methods: a method by affinity chromatography suitable for each tag generally used when the protein is attached to a tag such as 6× histidine, GST, or a maltose-binding protein; or alternatively a method that will be described in detail in Examples below, that is, a method by ion exchange chromatography, when the protein according to the present invention is produced without such a tag. In addition, the method can also include a method combining gel filtration, hydrophobic chromatography, isoelectric chromatography, and so on.

The enzyme protein according to the present invention is allowed to act on a glycoprotein, oligosaccharide, or

polysaccharide, or the like, thereby transferring a certain sugar residue. Thus, the enzyme according to the present invention can be used in the modification of an oligosaccharide in a glycoprotein and the synthesis of saccharides. In addition, by administering this enzyme as an immunogen to an animal, an antigen against the enzyme can be created, which can in turn be used to measure the enzyme by immunoassay. Thus, the enzyme and the nucleic acid encoding the enzyme according to the present invention are useful for creating such an immunogen.

It is preferred that the expression vector of the present invention should be constructed so that the enzyme is easily isolated and purified as described above. When the enzyme is prepared by a genetic engineering technique using the expression vector according to the present invention that has been constructed to be expressed especially in the morphology of a fusion protein between a polypeptide having an enzyme activity and a labeling peptide, its isolation and purification would easily be performed.

[0053]

An example of the identification (labeling) peptide described above is a peptide having the function of allowing the easy secretion/separation/purification or detection of the enzyme according to the present invention from a grown product of a transformant by expressing the enzyme according to the present invention as a fusion protein between the identification peptide and a polypeptide having an enzyme activity bound together in the preparation of the enzyme by genetic

recombination. Examples of such an identification peptide include a peptide such as a signal peptide (peptide consisting of 15 to 30 amino acid residues, which is present in the N terminuses of many proteins and functions within a cell for sorting out a protein in intracellular transmembrane mechanisms: e.g., OmpA, OmpT, and Dsb), a protein kinase A, a protein A (protein having a molecular weight of approximately 42,000, which is a component of the cell wall of *Staphylococcus aureus*), a glutathion S-transferase, a His tag (sequence where 6 to 10 histidine residues are aligned and arranged), a myc tag (13-amino acid sequence derived from a cMyc protein), a FLAG peptide (analytical marker consisting of 8 amino acid residues), a T7 tag (which consists of the first 11 amino acid residues in a gene 10 protein), a S tag (which consists of 15 amino acid residues derived from pancreatic RNase A), a HSV tag, pelB (22-amino acid sequence in an *E. coli* outer membrane protein pelB), a HA tag (which consists of 10 amino acid residues derived from hemagglutinin), a Trx tag (thioredoxin sequence), a CBP tag (calmodulin-binding peptide), a CBD tag (cellulose-binding domain), a CBR tag (collagen-binding domain), β -lac/blu (β lactamase), β -gal (β galactosidase), luc (luciferase), HP-Thio (His-patch thioredoxin), HSP (heat shock peptide), Lny (laminin γ peptide), Fn (partial peptide of fibronectin), GFP (green fluorescent peptide), YFP (yellow fluorescent peptide), CFP (cyan fluorescent peptide), BFP (blue fluorescent peptide), DsRed and DsRed2 (red fluorescent peptides), MBP (maltose-binding peptide), LacZ (lactose operator), IgG (immunoglobulin

G), avidin, and a protein G, and any of these identification peptides may be used. Among them, especially the signal peptide, protein kinase A, protein A, glutathione S-transferase, His tag, myc tag, FLAG peptide, T7 tag, S tag, HSV tag, pelB or HA tag is preferred because the enzyme according to the present invention is expressed and purified more easily according to a genetic engineering approach. It is particularly preferable to obtain the enzyme according to the present invention as a fusion protein with the FLAG peptide (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) (SEQ ID NO: 6), because of considerably excellent handling ease. The FLAG peptide is highly antigenic, and it provides an epitope reversibly bound by a specific monoclonal antibody and allows the rapid assay and easy purification of a recombinant protein expressed. A murine hybridoma designated as 4E11 produces a monoclonal antibody that binds to the FLAG peptide in the presence of a certain divalent metal cation, as described in U.S. Patent No. 5,011,912 (incorporated herein by reference). The 4E11 hybridoma cell line is deposited in American Type Culture Collection under Accession No. HB 9259. The monoclonal antibody binding to the FLAG peptide is available from Eastman Kodak Co., Scientific Imaging Systems Division, New Haven, Connecticut.

[0054]

A basic vector capable of being expressed in a mammalian cell and yielding the enzyme according to the present invention as a fusion protein with the FLAG peptide is, for example, pFLAG-CMV-1 (manufactured by Sigma-Aldrich). Alternatively,

a vector capable of being expressed in an insect cell is exemplified by pFBIF (a vector in which a region encoding the FLAG peptide is incorporated into pFastBac (Invitrogen): see Examples below). However, those skilled in the art can select a suitable basic vector, judging from a host cell, a restriction enzyme, an identification peptide, and so on, used in the expression of the enzyme.

[0055]

(4) Antibody recognizing protein according to the present invention

In the present specification, an antibody immunoreactive to a glycosyltransferase protein encoded by the nucleic acid of the present invention is provided. Such an antibody specifically binds to the glycosyltransferase protein via the antigen-binding site of the antibody (in contrast to non-specific binding). Thus, the protein of SEQ ID NO: 2, or a fragment, mutant, or fusion protein thereof, or the like, as described above may be used as an "immunogen" for producing an antibody immunoreactive thereto. To be more specific, the protein, the fragment, mutant, and fusion protein thereof, and so on, contain an antigenic determinant or epitope for eliciting the formation of an antibody. The antigenic determinant or epitope may be either linear or conformational (interrupted). The antigenic determinant or epitope may be identified by any method known in the art.

Thus, one aspect of the present invention relates to an antigenic epitope of a glycosyltransferase protein encoded by

the nucleic acid of the present invention. Such an epitope is useful for creating an antibody, especially a monoclonal antibody, as more fully described below. Furthermore, the epitope of the glycosyltransferase protein according to the present invention may be used in assay and as a research reagent for purifying an antibody specifically bound with the epitope from a substance such as a supernatant derived from polyclonal serum or a cultured hybridoma. Such an epitope or a mutant thereof can be produced using a technique known in the art such as solid phase synthesis and chemical or enzymatic cleavage of a protein, or using a recombinant DNA technique.

For an antibody likely to be induced by the glycosyltransferase protein, both polyclonal and monoclonal antibodies can be prepared by a routine technique even if the whole protein or a portion thereof is isolated or the epitope is isolated. See, e.g., Kennet et al., ed., *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, New York, 1980.

[0056]

The present specification is also directed to a hybridoma cell line that produces a monoclonal antibody specific to the glycosyltransferase protein of the present invention. Such a hybridoma can be produced and identified by a routine technique. One method for producing such a hybridoma cell line comprises: immunizing an animal with the glycosyltransferase protein; collecting spleen cells from the immunized animal; fusing the spleen cells with myeloma cell lines to thereby produce hybridoma

cells; and identifying a hybridoma cell line producing a monoclonal antibody that binds to the enzyme. The monoclonal antibody can be collected by a routine technique.

[0057]

The monoclonal antibody according to the present invention includes a chimeric antibody, for example, a humanized murine monoclonal antibody. When such a humanized antibody is prepared by a known technique and the antibody is administered to a human, an advantage of reduced immunogenicity may be provided.

An antigen-binding fragment of an antibody, which can be produced by a routine technique, is also encompassed by the present invention. Examples of such a fragment include, but are not limited to, Fab and $F(ab')_2$. An antibody fragment and a derivative produced by a genetic engineering technique are also provided.

The antibody according to the present invention can be used in assay for detecting the presence of the glycosyltransferase protein or a fragment thereof either *in vitro* or *in vivo*. The antibody can also be employed when the polypeptide of the present invention or a fragment thereof is purified by immunoaffinity chromatography.

[0058]

Furthermore, the use of a binding partner, for example, an antibody capable of blocking the binding of the glycosyltransferase protein to an acceptor substrate allows the inhibition of a biological activity generated from such binding. Such a blocking antibody may be identified using any suitable

assay method such as the test of antibody for the ability to inhibit the binding of the protein to a certain cell in which an acceptor substrate is being expressed. Alternatively, the blocking antibody can be identified in assay for the ability to inhibit biological influence arising from the enzyme according to the present invention bound with a binding partner for a target cell.

By using such an antibody an *in vitro* method or administering the antibody *in vivo*, a biological activity mediated by an entity producing the antibody can be inhibited. Thus, it is possible to treat disorders caused or deteriorated (directly or indirectly) by the interaction between the glycosyltransferase protein of the present invention and a binding partner. The therapy involves administering, to a mammal *in vivo*, the blocking antibody in an effective amount for inhibiting a binding partner-mediated biological activity. In general, a monoclonal antibody is preferred for use in such therapy. In one aspect, an antigen-binding antibody fragment is used.

Hereinafter, the present invention will be described more fully with reference to examples. However, the present invention is not intended to be limited to examples described below.

[0059]

[Examples]

Example 1

Cloning of DNA of the present invention

BLAST search using the sequence of a

β 1,3-N-acetylglucosaminyltransferase, a glycosyltransferase known in the art, as a query sequence was carried out and as a result, a genome sequence was found as a sequence having homology.

RNA was extracted from a colorectal cancer cell line colo 205 with RNeasy Mini Kit (manufactured by Qiagen) to synthesize single strand DNA by an oligo(dT) method using Super-Script First-Strand Synthesis System (manufactured by Invitrogen). This DNA was used as a template to carry out PCR with a 5' primer (SEQ ID NO: 3) and a 3' primer (SEQ ID NO: 4). PCR conditions comprised 25 cycles, each cycle having 94°C for 30 seconds, 65°C for 1 minute, and 72°C for 1 minute. A DNA fragment obtained by PCR has, as restriction sites, HindIII on the 5' side of the initiation codon of ORF and EcoRI on the 3' side of the stop codon thereof.

This DNA fragment and pBluescript(R) II SK(-) (manufactured by TOYOBO) were individually treated with restriction enzymes HindIII and EcoRI, with which a reaction solution was then mixed, followed by ligation reaction to thereby introduce the ORF of the G9 into the pBluescript(R) II SK(-). The reaction solution was purified by an ethanol precipitation method and then mixed with a competent cell (E. coli DH5 α). The mixture was subjected to a heat shock method (42°C, 30 sec.) and seeded to a LB agar medium containing IPTG and X-gal. The next day, a single white colony was further cultured to collect plasmid DNA.

The collected plasmid DNA was confirmed to contain the nucleic acid sequence of interest and the nucleotide sequence

was determined (SEQ ID NO: 1). A predicted open reading frame (ORF) in that nucleotide sequence is 1194 bp, and its predicted amino acid sequence consists of 397 amino acids (SEQ ID NO: 2). The predicted amino acid sequence has a hydrophobic amino acid region characteristic of a glycosyltransferase at its N terminus. Those consisting of the nucleic acid sequence and the amino acid sequence were designated as G9.

[0060]

The pBluescript(R) II SK (-) into which the G9 is incorporated is a multifunctional vector developed for carrying out cloning procedures and sequencing procedures more conveniently and has various improvements in addition to the function of conventional pUC and M13 vectors. Because a multi-cloning site is contained in a LacZ gene as with the pUC vector, the plasmid incorporating an insert therein is transformed into E. coli having the genotype of lacZ Δ M15 such as XL1-Blue MRF' and JM109, which in turn forms a white colony in a plate supplemented with IPTG/X-gal. Thus, the presence or absence of the insert can be easily assessed. Moreover, because the multi-cloning site has a polylinker consisting of 21 restriction sites, the range of choices for restriction enzymes used are extended when a deletion mutant is created by Exo/Mung System. The incorporated G9 gene can be adjusted in its expression within E. coli having lacI^q mutation through LacZ operator/promoter, and the E. coli is allowed to produce a protein of interest by the addition of IPTG to a medium. Furthermore, because T3 and T7 promoters are present on both sides of that

multi-cloning site, it is possible to create RNA probes with these promoters. BssHII sites are present on both sides of each of those promoters and can be utilized to cut out inserted DNA together with the promoter sequences. Using the probes from both of the promoters, gene mapping can be carried out. Because such a vector contains the replication origin of an f1 phage, single-stranded DNA is produced by the infection of a VCSM13 or R408 helper phage and can be used in sequencing and Site Specific Mutagenesis. An antisense strand is rescued by the infection of the helper phage.

[0061]

Example 2

Expression level of DNA of the present invention in human colorectal cancer tissue

The expression level of the G9 gene in normal and colorectal cancer tissues from identical patients were compared using a quantitative real-time PCR method.

The quantitative real-time PCR method is a method that combines sense and antisense primers with a fluorescently-labeled probe in PCR. In amplification by PCR, the fluorescent label of the probe comes off and shows fluorescence. Fluorescent intensity is amplified in correlation with the amplification of a gene and as such, used as an indicator to conduct quantification.

[0062]

RNA was extracted from human colorectal cancer tissues and normal and colorectal cancer tissues from identical patients

with RNeasy Mini Kit (manufactured by Qiagen) to synthesize single strand DNA by an oligo(dT) method using Super-Script First-Strand Synthesis System (manufactured by Invitrogen). This DNA was used as a template to carry out quantitative real-time PCR with ABI PRISM 7700 (manufactured by Applied Biosystems Japan) using a 5' primer (SEQ ID NO: 5), a 3' primer (SEQ ID NO: 6), and a TaqMan probe (SEQ ID NO: 7). PCR conditions comprised reaction at 50°C for 2 minutes and 95°C for 10 minutes, followed by cycles repeated 50 times, each cycle having 95°C for 15 seconds and 60°C for 1 minute. The obtained measurement values were divided by a value from β -actin as an internal standard gene quantified using a kit manufactured by Applied Biosystems Japan Ltd., in order to correct variations among individuals. Comparison was made between the measurement values of the human colorectal cancer tissues and those of the normal and colorectal cancer tissues from identical patients.

The result has demonstrated that the transcript from the DNA of the present invention is not present or is too negligible to measure in the non-cancerous tissues and that the transcript from the DNA of the present invention is significantly present in the cancerous tissues (see Table 3).

[0063]

[Table 3]

Table 3

Patient No.	Normal tissue	Cancer tissue
Patient 1	0	0.0052
Patient 2	0	0.0004
Patient 3	0	0.0023

Patient 4	0	0.0012
Patient 5	0	0.0018
Patient 6	0	0.0028
Patient 7	0	0.0007
Patient 8	0	0.0057
Average	0.0000000	0.0025125

[0064]

Expression level of DNA of the present invention in human peripheral blood

The expression level of the G9 gene in peripheral blood of normal individuals and patients with colorectal cancer was compared using a quantitative real-time PCR method.

Blood was collected into a PAXgene blood RNA tube (manufactured by PreAnalytix) from healthy volunteers and patients with colorectal cancer. After the blood was mixed by inversion with reagents in the tube and reacted at room temperature for 24 hours, RNA was extracted with a PAXgene blood RNA kit (manufactured by PreAnalytix). Using Super-Script First-Strand Synthesis System (manufactured by Invitrogen), cDNA was synthesized with accompanying random primers. This DNA was used as templates to carry out quantitative real-time PCR with ABI PRISM 7700 (manufactured by Applied Biosystems Japan) using a 5' primer (SEQ ID NO: 4), a 3' primer (SEQ ID NO: 5), and a TaqMan probe (SEQ ID NO: 6). PCR conditions comprised reaction at 50°C for 2 minutes and 95°C for 10 minutes, followed by cycles repeated 50 times, each cycle having 95°C for 15 seconds and 60°C for 1 minute. The obtained measurement values were divided by a value from β -actin as an internal standard gene quantified using a kit manufactured by Applied Biosystems

Japan Ltd., in order to correct variations among individuals. Comparison was made between the normal individuals and the patients with colorectal cancer.

[0065]

The result has demonstrated that the transcription level of the DNA of the present invention in the peripheral blood from the patients with colorectal cancer is significantly greater than that in the peripheral blood from the normal individuals. When patients with colorectal cancer having the measurement value that exceeds the average measurement value of normal individuals + (standard deviation x2) were assessed to be positive, the positive rate of the patients with colorectal cancer was 67% (see Table 4).

[0066]

[Table 4]

Table 4

No.	Normal individual	Patient with colorectal cancer	Assessment
1	90	121	Positive
2	107	68	Negative
3	82	199	Positive
4	81	418	Positive
5	87	123	Positive
6		92	Negative
7		196	Positive
8		86	Negative
9		473	Positive
10		267	Positive
11		110	Positive
12		46	Negative
Average	89.4	183.3	
Standard deviation	9.4	132.1	

[0067]

Example 3

Expression of isolated full-length G9

The plasmid DNA where the G9 was incorporated into the pBluescript(R) II SK(-) and pcDNA3.1 (manufactured by Invitrogen) were individually treated with restriction enzymes HindIII and EcoRI, with which a reaction solution was then mixed, followed by ligation reaction to thereby introduce the ORF of the G9 into the pcDNA3.1(+). The reaction solution was purified by an ethanol precipitation method. Then, by investigating the sequence, the G9 was confirmed to be introduced into the pcDNA3.1(+) and this was designated as pcDNA3.1(+)-G9. The pcDNA3.1(+)-G9 was mixed with a competent cell (E. coli DH5 α). The mixture was subjected to a heat shock method (42°C, 45 sec.) and seeded to a LB agar medium containing ampicillin. The next day, a single colony was further cultured to collect plasmid DNA. The collected plasmid DNA was confirmed to contain the nucleic acid sequence of interest and the nucleotide sequence was determined.

[0068]

The pcDNA3.1(+/-) is an expression vector for a broad variety of mammalian cells. It is a vector for forward transcription, in which a sequence likely to form the secondary structure of RNA is removed from a multi-cloning site (MCS) sequence by improving conventional pcDNA3.1 for enhancing expression level. The pcDNA3.1(+/-) has the enhancer/promoter of CMV and allows a high level of expression. RNA is stabilized by a polyadenylated

signal and a transcription termination sequence. Because there exists a SV40 origin, the pcDNA3.1(+/-) can be replicated in a cell in which a SV40 Large T antigen is being expressed. An ampicillin-resistant gene is introduced therein for selection in *E. coli*. A neomycin-resistant gene is also introduced therein for selection for producing a stable strain in a mammalian cell.

[0069]

HCT15 cells, human colorectal cancer-derived cell lines, were used to conduct procedures below for creating G9-expressing stable strains. The HCT15 cells were suspended at 2×10^6 cells in 10 ml of a RPMI-1640 medium containing 10% fetal bovine serum but no antibiotic, then seeded to a 10-cm dish, and cultured in a CO₂ incubator at 37°C for 16 hours. The plasmid DNA (20 ng) of the pcDNA3.1(+)-G9 and 30 µl of Lipofectamine 2000 (manufactured by Invitrogen) were mixed with 1.5 ml of OPTI-MEM (manufactured by Invitrogen), respectively, and incubated at room temperature for 5 minutes. Further, these two solutions were gradually mixed and incubated at room temperature for 20 minutes. This mixture solution was added dropwise to the dish and cultured in a CO₂ incubator at 37°C for 48 hours. The cells were subcultured by a standard method. On this occasion, RPMI-1640 (manufactured by Invitrogen) was used as a medium, to which fetal bovine serum, and penicillin (manufactured by Invitrogen), streptomycin (manufactured by Invitrogen), and Geneticin (neomycin; manufactured by Invitrogen) as antibiotics were added. Because cells in which the pcDNA3.1(+)-G9 has not been introduced are allowed to die out by the addition of Geneticin,

continued culture results in the survival of only cells in which the pcDNA3.1(+)-G9 has been introduced. These cells were used as G9-expressing stable strains.

[0070]

Expression of G9 recombinant protein in mammalian cell line

For obtaining a G9 recombinant protein, the G9 was expressed in a human kidney-derived cell line 293T. Only the expression of an active region from the 105th amino acid to the C terminus in SEQ ID NO: 2 which relatively retains homology to at least β 1,3GlcNAc transferase and β 1,3Gal transferase may be adequate for investigating a function. However, here, it has been decided to express two predicted active regions from the 24th amino acid and the 33rd amino acid to the C terminus in the G9.

[0071]

Using, as a template, the plasmid DNA where the G9 was incorporated into the pBluescript(R) II SK(-), PCR reaction was carried out with each of 5' primers (SEQ ID NOs: 8 and 9) and a 3' primer (SEQ ID NO: 10) to obtain a DNA fragment of interest. The PCR method comprised 25 cycles, each cycle having 94°C for 30 seconds, 65°C for 1 minute, and 72°C for 1 minute. Then, a PCR product was subjected to agarose gel electrophoresis. The gel was cut out by a gel excision method to isolate the PCR product by a standard method. This PCR product had HindIII on the 5' side and EcoRI on the 3' side as restriction sites. This DNA fragment and pFLAG-CMV3 were individually treated with restriction enzymes HindIII and EcoRI, with which a reaction solution was then mixed, followed by ligation reaction to thereby

introduce the DNA fragment into the pFLAG-CMV3. The reaction solution was purified by an ethanol precipitation method and then mixed with a competent cell (E. coli DH5 α). The mixture was subjected to a heat shock method (42°C, 45 sec.) and seeded to a LB agar medium containing ampicillin.

[0072]

The next day, the DNA of interest in the resulting colony was directly confirmed by PCR. After the DNA sequence was confirmed by sequencing for additional confirmation, the vector (pFLAG-CMV3) was extracted and purified.

Human kidney cell-derived cell line 293T cells were suspended at 2×10^6 cells in 10 ml of a DMEM medium containing 10% fetal bovine serum but no antibiotic, then seeded to a 10-cm dish, and cultured in a CO₂ incubator at 37°C for 16 hours. The pFLAG-CMV3-G9 (20 ng) and 30 μ l of Lipofectamine 2000 (manufactured by Invitrogen) were mixed with 1.5 ml of OPTI-MEM (manufactured by Invitrogen), respectively, and incubated at room temperature for 5 minutes. Further, these two solutions were gradually mixed and incubated at room temperature for 20 minutes. This mixture solution was added dropwise to the dish and cultured in a CO₂ incubator at 37°C for 48 hours.

[0073]

With 10 ml of the resulting culture supernatant, NaN₃ (0.05%), NaCl (150 ml), CaCl₂ (2 ml), anti-FLAG M1 affinity gel (manufactured by Sigma) (100 μ l) were mixed and stirred overnight at 4°C. The next day, the mixture was centrifuged (3000 rpm, 5 min, 4°C) to collect a pellet to which 900 μ l of 2 ml CaCl₂/TBS

was in turn added. The mixture was centrifuged again (2000 rpm, 5 min, 4°C), and the resulting pellet was floated in 200 µl of 1 ml CaCl₂/TBS and used as a sample (G9 enzyme solution) for activity measurement. A portion of this was subjected to electrophoresis by SDS-PAGE and subsequently to western blotting with anti FLAG-M2 peroxidase (manufactured by Sigma), to confirm the expression of the G9 protein of interest. As a result, a band was detected at the position of approximately 45 kDa, and the expression was therefore confirmed.

[0074]

Expression of G9 recombinant protein in an insect cell line

For obtaining a G9 recombinant protein, the G9 was expressed in an insect cell. Only the expression of an active region from the 105th amino acid to the C terminus in SEQ ID NO: 2 which relatively retains homology to at least β 1,3GlcNAc transferase and β 1,3Gal transferase may be adequate for investigating a function. However, here, it has been decided to express a predicted active region from the 36th amino acid to the C terminus in the G9.

Using, as a template, the plasmid DNA where the G9 was incorporated into the pBluescript(R) II SK(-), PCR reaction was carried out with a 5' primer (SEQ ID NO: 11) and a 3' primer (SEQ ID NO: 12) to obtain a DNA fragment of interest. The PCR method comprised 25 cycles, each cycle having 94°C for 30 seconds, 65°C for 1 minute, and 72°C for 1 minute. Then, a PCR product was subjected to agarose gel electrophoresis. The gel was cut out by a gel excision method to isolate the PCR product by a

standard method. The PCR product thus isolated was incorporated into pDONRTM 201 (manufactured by Invitrogen) by BP clonase reaction to create an "entry clone".

[0075]

Reaction was carried out by incubating 2 μ l of the PCR product, 1 μ l (150 ng) of the pDONR 201, 2 μ l of a BP reaction buffer, 3 μ l of a Tris-EDTA buffer (pH 8.0; hereinafter, also abbreviated to "TE"), and 2 μ l of BP clonase mix at 25°C for 1 hour. The mixture was then supplemented with 1 μ l of a proteinase K (manufactured by Kaken Pharmaceutical) and incubated at 37°C for 10 minutes to terminate the reaction. The reaction mixture solution (11 μ l) was mixed with 100 μ l of a competent cell (E. coli DH5 α), then transformed by a heat shock method, and seeded to a LB plate containing kanamycin. The next day, a colony was collected to confirm the introduction of the DNA of interest and its nucleotide sequence by PCR. The vector in which the DNA was inserted (pDONR-G9) was extracted and purified according to a standard method. The nucleotide sequence of the DNA inserted in this vector was confirmed to contain the nucleotide sequence described in SEQ ID NO: 1.

[0076]

Preparation of expression clone

The above-described entry clone has attL sites at both ends of the inserted site that are recombination sites when a λ phage is excised from E. coli. By mixing the entry clone with a LR clonase (mixture of λ phage recombinases Int, IHF, and Xis) and a destination vector (which has attR), the inserted site was

transferred to the destination vector to generate an expression clone.

With 1 μ l of the entry clone (pDONR-G9), 0.5 μ l (75 ng) of the destination vector (pFBIF), 2 μ l of a LR reaction buffer, 4.5 μ l of TE, and 2 μ l of LR clonase mix (mixture solution of λ phage recombinases Int, IHF, and Xis) were incubated at 25°C for 1 hour. The mixture was then supplemented with 1 μ l of a proteinase K (manufactured by Kaken Pharmaceutical) and incubated at 37°C for 10 minutes to terminate the reaction (this recombination reaction yields pFBIF-G9). The pFBIF was obtained by inserting an Igk signal sequence and a FLAG peptide for purification into pFastBac1 (manufactured by Invitrogen) according to a standard method. For further inserting the Gateway sequence (attR) into the pFBIF, Gateway Vector Conversion System (manufactured by Invitrogen) was used to insert a conversion cassette. This conversion cassette is a cassette for altering an expression vector to a destination vector and has an attR recombination site, a chloramphenicol-resistant gene, and a ccdB gene encoding a protein that inhibits an E. coli DNA gyrase. The Igk signal sequence was inserted for rendering an expressed protein secretory, while the FLAG tag was inserted for facilitating purification.

[0077]

The reaction mixture solution (11 μ l) containing the pFBIF-G9 and 100 μ l of a competent cell E. coli DH5 α were mixed and transformed by a heat shock method, and the resulting recombinant DH5 α was seeded to a LB medium containing ampicillin

and then cultured. After 24-hour culture, a colony was collected, and the plasmid (pFBIF-G9) was extracted and purified by QIAprep Spin Miniprep Kit (manufactured by Qiagen). A PCR method was used to confirm the insertion of the DNA of interest.

Preparation of bacmid by Bac-to-Bac system (manufactured by Invitrogen)

Subsequently, using a Bac-to-Bac system (manufactured by Invitrogen), recombination was conducted between the pFBIF-G9 and a bacmid to insert the G9 sequence into the bacmid capable of proliferation in an insect cell. This system is a system that utilizes the recombination site of Tn7 and allows the incorporation of the gene of interest (G9) into the bacmid through a recombination protein produced by a helper plasmid only by introducing, into bacmid-containing E. coli (E. coli DH10Bac™), pFastBac in which the gene of interest is inserted (i.e., pFBIF-G9). Moreover, the bacmid contains a LacZ gene and is selectable by classical colony colors (blue (without insertion) to white (with insertion)).

That is, 50 µl of the above-described purified vector (pFBIF-G9) and 50 µl of a competent cell (E. coli DH10Bac) were mixed, then transformed by a heat shock method, and seeded to a LB medium containing kanamycin, gentamicin, tetracycline, 5-bromoindolyl-β-D-galactopyranoside (Bluo-gal), and isopropyl-β-D-thiogalactopyranoside (IPTG). After 24 hours, an independent white colony where the DNA of interest is inserted into the bacmid was collected and further cultured to collect

the bacmid according to a standard method.

[0078]

Introduction of bacmid into insect cell

The insertion of the DNA of interest into the collected bacmid was confirmed according to a standard method, and the bacmid was transfected into an insect cell (Sf21; manufactured by Invitrogen). That is, a Sf900SFM medium (manufactured by Invitrogen) containing an antibiotic was added to the Sf21 cells at 9×10^5 cells/2 ml which was then allowed to adhere to a 35-mm Petri dish at 27°C for 1 hour. After the cells were confirmed to adhere to the Petri dish, the culture solution was aspirated. The cells were supplemented and incubated at 27° for 5 hours with a culture solution where 800 µl of Sf900II (manufactured by Invitrogen) was added to a solution of lipid-DNA complexes (solution obtained by gently mixing and incubating, at room temperature for 30 minutes, A Solution (mixture of 5 µl of the above-described bacmid added to 100 µl of Sf-900SFM) and B Solution (mixture of 6 µl of Cellfectin Reagent (manufactured by Invitrogen) added to 100 µl of Sf-900SFM)). Then, the medium was removed, and the cells were supplemented with 2 ml of a Sf900SFM medium containing an antibiotic and incubated 27°C for 72 hours. After culture, the cells were liberated by pipetting. The cells and the culture solution were collected and centrifuged at 1000×g for 10 minutes to collect a supernatant (this supernatant was used as a "primary virus solution").

[0079]

Further, 1×10^7 Sf21 cells/20 ml of Sf-900SFM (containing

an antibiotic) were added to a T75 culture flask and incubated at 27°C for 1 hour. After the adhesion of the cells to the flask, 800 µl of the primary virus solution was added and cultured at 27°C for 48 hours. After culture, the cells were liberated by pipetting. The cells and the culture solution were collected and centrifuged at 1000×g for 10 minutes to collect a supernatant (this supernatant was used as a "secondary virus solution").

Further, 1×10^7 Sf21 cells/20 ml of Sf-900SFM (containing an antibiotic) were added to a T75 culture flask and incubated at 27°C for 1 hour. After the adhesion of the cells to the flask, 1000 µl of the secondary virus solution was added and cultured at 27°C for 84 hours. After culture, the cells were liberated by pipetting. The cells and the culture solution were collected and centrifuged at 1000×g for 10 minutes to collect a supernatant (this supernatant was used as a "tertiary virus solution").

In addition, 100 µl of Sf-900SFM (containing an antibiotic) containing Sf21 cells at a concentration of 6×10^5 cells/ml and subsequently 1 ml of the tertiary virus solution were added to a 100-ml spinner flask and cultured at 27°C for 96 hours. After culture, the cells and the culture solution were collected and centrifuged at 1000×g for 10 minutes to collect a supernatant (this supernatant was used as a "quaternary virus solution").

NaN₃, NaCl, and CaCl₂ were added to 10 ml of the quaternary virus solution. The final concentration is set to 0.05% for NaN₃, to 150 mM for NaCl, and to 2 mM for CaCl₂. To the mixture solution, 50 µl of anti-FLAG M1 antibody affinity gel (manufactured by Sigma) was added and gently mixed by inversion

at 4°C for 16 hours. Following centrifugation (1000×g, 3 min. 4°C) to remove the resulting supernatant, the affinity gel was washed twice with TBS (Tris-buffered saline, pH 7.4) containing 1 mM CaCl₂. After washing, the affinity gel was suspended in 200 µl of TBS (pH 7.4) containing 1 mM CaCl₂, and this suspension was used as a G9 enzyme solution for activity measurement.

[0080]

Example 5

Analysis for enzyme activity

The result of comparison with other glycosyltransferases known in the art on the basis of the amino acid sequence described in SEQ ID NO: 2 has suggested that the G9 is classified into transferases in light of, for example, the conservation of a sequence in the C-terminal region considered as an active site. Therefore, for example, UDP-GlcNAc can be used as a GlcNAc donor substrate to confirm the enzyme activity of the enzyme solution containing the G9 polypeptide, which has been obtained in Example 4 above.

[0081]

For example, GalNAcα1-Bz, GalNAcβ1-pNp, GlcNAcα1-Bz, GlcNAcβ1-Bz, Galα1-pNp, Galβ1-oNp, Xylβ1-pNp, Fucα1-pNp, Manα1-Bz, ManNAcα1-Bz, Galβ1-4Glcβ1-Bz (Sigma Corp.) and Galβ1-4GlcNAcα1-pNp (Toronto Research Chemicals Inc.) were used as GlcNAc acceptor substrates. The activity can be confirmed by preparing a reaction solution so to include 10nmol of each of above substrates at a final concentration.

For example, the reaction solution comprises 20 µl in total

of sodium cacodylate (pH of each experimental system is prepared 6.6 or 7.4), 0.4% Triton (trademark) CF-54 (Sigma Corp.) at a final concentration, 480 μ M UDP-GlcNAc and 175 nCi UDP- 14 C]GlcNAc at a final concentration, 20 mM $MgCl_2$, $CoCl_2$ or $CuCl_2$ at a final concentration, 10.1 μ l G9 enzyme solution, and distilled water.
[0082]

For example, the reaction solution is incubated at 37°C for 16 hours and then, added 200 μ l of distilled water, removed insoluble fragment by centrifugation (100 rpm, 10 min) to obtain supernatant fragment. The fragment may be loaded onto Sep-Pak plus C18 Cartridge (Waters: it is in advance equilibrated by washing with 1 ml methanol once and 1 ml distilled water twice) , and the specific acceptor substrates and reaction products can be adsorbed in the cartridge. After the cartridge was washed twice with 1 ml of pure water, the adsorbed specific acceptor substrates and reaction products were eluted with 1 ml of methanol. Following the mixing the eluted solution with a liquid scintillator (ACSII; manufactured by Amersham Biosciences), the amount of radiation from the product can be measured with a scintillation counter.

As a result, for example, the enhancement of an enzyme activity under the condition that a divalent metal cation is chelated by EDTA at the optimal pH, for example, manganese ion (Mn^{2+}) is shown.

[0083]

SEQUENCE LISTING

<110> National Institute of Advanced Industrial Science and Technology

Fujirebio Incorporated

<120> Novel glycosyl transferase, a DNA encoding the same, and a meth

od for detection for cancer

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ccctcggggc 120

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cctctccacc 180

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Lys Ala Tyr Pro Ser Pro Arg Gly Thr Pro Pro Ser Pro Thr Pro Ala
35 40 45

Asn Pro Glu Pro Thr Leu Pro Ala Asn Leu Ser Thr Arg Leu Gly Gln
50 55 60

Thr Ile Pro Leu Pro Phe Ala Tyr Trp Asn Gln Gln Gln Trp Arg Leu
65 70 75 80

Gly Ser Leu Pro Ser Gly Asp Ser Thr Glu Thr Gly Gly Cys Gln Ala
85 90 95

Trp Gly Ala Ala Ala Ala Thr Glu Ile Pro Asp Phe Ala Ser Tyr Pro
100 105 110

Lys Asp Leu Arg Arg Phe Leu Leu Ser Ala Ala Cys Arg Ser Phe Pro
115 120 125

Gln Trp Leu Pro Gly Gly Gly Gly Ser Gln Val Ser Ser Cys Ser Asp
130 135 140

Thr Asp Val Pro Tyr Leu Leu Leu Ala Val Lys Ser Glu Pro Gly Arg
145 150 155 160

Phe Ala Glu Arg Gln Ala Val Arg Glu Thr Trp Gly Ser Pro Ala Pro
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Gly Ile Arg Leu Leu Phe Leu Leu Gly Ser Pro Val Gly Glu Ala Gly
180 185 190

Pro Asp Leu Asp Ser Leu Val Ala Trp Glu Ser Arg Arg Tyr Ser Asp
195 200 205

Leu Leu Leu Trp Asp Phe Leu Asp Val Pro Phe Asn Gln Thr Leu Lys
210 215 220

Asp Leu Leu Leu Leu Ala Trp Leu Gly Arg His Cys Pro Thr Val Ser
225 230 235 240

Phe Val Leu Arg Ala Gln Asp Asp Ala Phe Val His Thr Pro Ala Leu
245 250 255

Leu Ala His Leu Arg Ala Leu Pro Pro Ala Ser Ala Arg Ser Leu Tyr
260 265 270

Leu Gly Glu Val Phe Thr Gln Ala Met Pro Leu Arg Lys Pro Gly Gly
275 280 285

Pro Phe Tyr Val Pro Glu Ser Phe Phe Glu Gly Gly Tyr Pro Ala Tyr
290 295 300

Ala Ser Gly Gly Gly Tyr Val Ile Ala Gly Arg Leu Ala Pro Trp Leu
305 310 315 320

Leu Arg Ala Ala Ala Arg Val Ala Pro Phe Pro Phe Glu Asp Val Tyr
325 330 335

Thr Gly Leu Cys Ile Arg Ala Leu Gly Leu Val Pro Gln Ala His Pro

340 345 350

Gly Phe Leu Thr Ala Trp Pro Ala Asp Arg Thr Ala Asp His Cys Ala

355 360 365

Phe Arg Asn Leu Leu Leu Val Arg Pro Leu Gly Pro Gln Ala Ser Ile

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Arg Leu Trp Lys Gln Leu Gln Asp Pro Arg Leu Gln Cys

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[Name of Document] Abstract

[Abstract]

[Problems that the Invention to Solve]

A novel nucleic acid and method of testing canceration using the nucleic acid is provided.

A tumor marker nucleic acid of the present invention is concerned with a nucleic acid hybridizing under stringent conditions to a nucleotide sequence described in SEQ ID NO: 1 or a complementary nucleotide sequence thereof. A method of testing canceration of the present invention is a method comprising (a) measuring the transcription level of the nucleic acid in the biological sample using a nucleic acid hybridizing with a nucleotide sequence described in SEQ ID NO: 1 or a complementary nucleotide sequence thereof under stringent conditions (b) diagnosing a biological sample as being cancerous when the transcription level of the nucleic acid in the biological sample significantly exceeds that in a normal biological sample as a control.

[Selected Drawing] None